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(54) Title: PRESERVATIVE SYSTEM

(57) Abstract: Use of an essential oil to potentiate the activity of a biocide, wherein the essential oil has a specific gravity of at least 0.89 and a dielectric constant of 1 to 20.

Preservative System

The present invention relates to new preservative systems and their use in liquid composition, in particular pharmaceutical compositions.

Preservatives are well known. Preservatives are intended to preserve a product, such as a food or a pharmaceutical, against microbial growth and product spoilage. Preservatives usually have good selective toxicity. Thus, they are typically safe for topical application and/or ingestion. Typically, such preservatives include biocides, which inhibit the growth of microorganisms, such as bacteria. Known biocides include phenolic compounds, such as esters of p-hydroxy benzoic acid. Such esters are known as parabens, examples of which are methyl paraben (methyl ester of p-hydroxy benzoic acid), ethyl paraben (ethyl ester of p-hydroxy benzoic acid), propyl paraben (propyl ester of p-hydroxy benzoic acid) and butyl paraben (butyl ester of p-hydroxy benzoic acid).

In WO 99/09836, it was found that certain plant oils could be used to enhance the antimicrobial activity of parabens. In particular, it was found that the antimicrobial activity of methyl paraben could be synergistically enhanced in the presence of fennel oil, anise oil and basil oil. Estragol, trans-anethole and fenchone were also found to synergistically enhance the antimicrobial activity of methyl paraben.

Without wishing to be bound by any theory, the present inventors have discovered a relationship between the physical properties of essential oil and its ability to potentiate the antimicrobial activity of a biocide. Specifically, it is believed that essential oil having a

relatively high specific gravity and a relatively low dielectric constant can be used to potentiate the antimicrobial activity of a biocide, such as a paraben.

We present, as a feature of the invention, the use of an essential oil to potentiate the activity of a biocide, wherein the essential oil has a specific gravity of at least 0.89 and a dielectric constant of 1 to 20, and, preferably, wherein the essential oil does not consist of a single essential oil selected from fennel oil, anise oil, basil oil, fenchone, trans-anethole and estragol.

We present, as a further feature of the invention, the use of an essential oil as a preservative in a liquid composition comprising a biocide, wherein the essential oil has a specific dielectric of at least 0.89 and a gravity constant of 1 to 20, and, preferably, wherein the essential oil does not consist of a single essential oil selected from fennel oil, anise oil, basil oil, fenchone, trans-anethole and estragol.

As a further feature we present as a feature of the invention, a pharmaceutical composition comprising:

a) an alginate,

b) a bicarbonate and/or a carbonate,

c) a biocide, and

d) an essential oil,

wherein the essential oil has a specific gravity of at least 0.89 and a dielectric constant of 1 to 20, and, preferably, wherein the essential oil does not consist of a single essential oil selected from fennel oil, anise oil, basil oil, fenchone, trans-anethole and estragol.

It is believed that the antimicrobial activity of the biocide may be enhanced by the presence of an essential oil having a specific gravity of at least 0.89 and a dielectric

constant of 1 to 20. Thus, a further aspect of the present invention provides the use of an essential oil to potentiate the activity of a biocide, wherein the essential oil has a specific gravity of at least 0.89 and a dielectric constant of 1 to 20, and wherein the essential oil does not consist of a single essential oil selected from fennel oil, anise oil, basil oil, fenchone, trans-anethole and estragol.

In a preferred embodiment, the antimicrobial activity of the biocide is synergistically enhanced by the essential oil. Thus, in this embodiment, the antimicrobial activity of the combination of the biocide and the oil is greater than the sum of the antimicrobial activity of the biocide in the absence of the oil and the antimicrobial activity of the oil in the absence of the biocide. For example, if a culture of bacteria is treated with the combination of the biocide and oil of the present invention, the number of bacteria eliminated in the sample after a predetermined period of time is preferably greater than the sum of i) the number of bacteria eliminated after the same predetermined period in an identical culture treated with the biocide in the absence of the oil and ii) the number of bacteria eliminated after the same predetermined period in another identical culture treated with the oil in the absence of the biocide.

Preferably, the essential oil has a specific gravity of greater than 0.890, more preferably, at least 0.990. The essential oil may have a specific gravity of 0.990 to 2.000, for example, 1.000 to 1.700, preferably, 1.010 to 1.500. In one embodiment, the specific gravity of the essential oil is 1.020 to 1.300, preferably, 1.030 to 1.250, most preferably, 1.170 to 1.200. Specific gravity may be measured using any suitable method. For example, conventional instruments,

such as a pycnometer may be used. Gravimetric analysis may also be employed.

The essential oil may have a dielectric constant of 2 to 18, preferably 3 to 12, more preferably 5 to 11, for example, 6 to 9. Dielectric constant is measured using any suitable method. For example, a parallel plate capacitor may be employed.

Any suitable essential oil may be employed in the composition of the claimed invention. For example, essential plant oils may be employed. For the avoidance of doubt, the term "essential oil" covers essential oils and the oily components of essential oils. A mixture of two or more oils may also be employed, particularly if the mixture has a specific gravity of at least 0.89 and a dielectric constant of 1 to 20.

Preferably, the oil has an aromatic component content of at least 30 %, more preferably at least 50%, even more preferably, at least 60 %, for example, at least 80%. The aromatic components are preferably water-insoluble. Preferably the essential oil is present in the liquid composition as an oil-in-water emulsion.

Preferably, the essential oil comprises at least one oil selected from wintergreen oil, clove oil, spearmint oil, carvone, methyl salicylate and eugenol. The oil may be a mixture of spearmint oil and wintergreen oil or a mixture of spearmint oil and clove oil or eugenol. When a mixture of spearmint oil and wintergreen oil is employed, the weight ratio of spearmint oil to wintergreen oil may be 10 - 90: 90 - 10, preferably, 30 - 70: 70 - 30, for example, 40 - 60: 60 - 40. When a mixture of spearmint oil and clove oil or eugenol is employed, the ratio of spearmint oil to clove oil

or eugenol may be 10 - 90: 90 - 10, preferably, 30 - 70: 70 - 30, for example, 40 - 60: 60 - 40.

In a further embodiment, the oil is a mixture of wintergreen oil and eugenol. The weight ratio of wintergreen oil to eugenol may be 10 - 90: 90 - 10, preferably, 30 - 70: 70 - 30, for example, 40 - 60: 60 - 40.

As mentioned above, preferably oils consisting of one of fennel oil, basil oil and anise oil are excluded from the scope of the invention. Similarly, oils consisting of one of estragol, trans-anethole and fenchone may also be excluded. For the avoidance of doubt, therefore, oils formed of 100% of one of estragol, trans-anethole and fenchone are also preferably excluded. Preferably, oils formed of at least 70 weight %, preferably, at least 80 weight %, more preferably, at least 90 weight %, for example, at least 95 weight % of one of estragol, trans-anethole and fenchone are also excluded.

According to a further embodiment of the invention, oils consisting of peppermint oil may also be excluded from the scope of the invention.

The oil may be present in an amount of from 0.01 to 5 volume % of the composition, preferably, from 0.05 to 2 volume %, more preferably, from 0.07 to 1 volume %. In one embodiment, the oil is present in an amount of 0.01 to 0.5, for example, 0.01 to 0.1 volume %.

The biocide employed in the composition may be an ester of p-hydroxy benzoic acid. Such esters are known as parabens. Examples of suitable parabens include methyl paraben, ethyl paraben, propyl paraben and butyl paraben (or their salts [e.g. soluble salts]). Mixtures of parabens may be employed. Preferably, methyl paraben and/or propyl

paraben are employed. Alternatively, the biocide may be bronopol, chlorocresol or an isothiazole.

The amount of biocide present in the composition may be 0.001 to 50 weight % of the composition, preferably, from 0.01 to 30 weight %.

The ratio of essential oil to biocide in the composition may be 1:20 to 20:1, preferably, from 1:10 to 10:1, and more preferably, from 1:5 to 5:1.

Typically, the composition is an aqueous composition comprising up to 99.99% water. For example, the aqueous composition may comprise from 60 to up to 99.99 weight % water, for example, from 70 to 98 weight % water. It may, however, be possible to provide the composition in the form of a concentrate. The concentrate may be substantially anhydrous or may contain water in an amount of 0.1 to 50 weight %, preferably, 10 to 30 weight %. Such a concentrate may be diluted with water or an aqueous composition before use.

The composition may also comprise a chelating agent. The chelating agent may enhance the interactions between the biocide and the bacterial wall by exposing more lipophilic surfaces on the bacterium. Suitable chelating agents include ethylene diaminetetraacetic acid (EDTA) or its derivatives (e.g. salts).

The composition is in the form of a liquid. Preferably, the liquid is pourable or in a form suitable for administration in the form of droplets or a spray.

The composition may be suitable for oral administration. Thus, the composition may be in the form of a mouthwash, a toothpaste or chewing gum.

The pharmaceutical composition may be suitable for the treatment of gingivitis, sore throat, cough, reflux

esophagitis, gastritis, dyspepsia or peptic ulceration, or for use as a sustained release or targeted delivery composition. The pharmaceutical composition may be in the form of an aqueous pourable liquid comprising an alginate and a bicarbonate and/or carbonate. When forming the pharmaceutical composition, the biocide and oil may be added to the pharmaceutical composition separately or in combination.

The biocide may be present in an amount from 0.001 to 5 weight % of the composition, preferably, from 0.01 to 2 weight %, more preferably, from 0.07 to 1 weight %, for example, from 0.1 to 0.2 weight %.

The composition may be used to inhibit the growth of a number of different bacteria. For example, the composition may be useful against *Ps aeruginosa, Enterobacter cloacae, Klebsiella oxytoca, Klebsiella pneumonia and Citobacter freundii.*

Example 1

In this example, compositions comprising methyl paraben, propyl paraben and a number of different oils were tested for their antimicrobial activity against the *Ps aeruginosa*. The following oils were tested: I) spearmint oil, ii) spearmint oil and eugenol (63.5:36.5)and iii) eugenol. A control sample C1) of spearmint oil and eugenol was also prepared (63.5:36.5). However, no parabens were included in the control.

To carry out the tests, the following procedure was adopted.

A culture of *Ps aeruginosa* was grown in Tryptone Soy Broth (TSB Oxoid) for 6 hours and spread onto Tryptone Soy

Agar (TSA Oxoid). The bacteria were left to multiply overnight at 37°C. The bacteria were then harvested, washed and re-suspended in HEPES buffer (pH=7.9).

9ml of parabens stock containing 0.150%w/v methyl paraben and propyl paraben was measured into a test tube. Specifically, 0.130%w/v methyl paraben and 0.020%w/v propyl paraben was employed.

7.8µl of the oil under test was added (using SGE 10µl micro-syringe) to the 9ml parabens stock. The test-tube was vortexed for 30 seconds and left to stand in a stirred water bath at 25°C for 20 minutes.

1ml of the bacteria suspended in HEPES buffer [N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid] was then added to the test tube. The test tube was then vortexed for 15 seconds and placed in the stirred water bath at 25°C.

At predetermined time intervals, the test tube was vortexed for 5 seconds and a 1ml sample removed. This sample was added to 9ml of a neutralizer comprising HEPES, Tween [1.11%w/v Tween ® 80 (polysorbate 80); laboratory grade, Product #560234H, Cas# 9005-65-6 (BDH, Poole, Dorset, UK)] and Lecithin [0.085% w/v Lechitin Soya Bean; Laboratory grade, Product # 298632A, Cas#8030-76-0]. The test tube was then returned to the 25°C stirred water bath for the next sample.

Each neutralized sample (20µl) was spread out onto a plate containing Tryptone Soy Agar (Oxoid) and incubated overnight at 30°C.

Figure 1 shows how the number of bacteria decreased over time. As can be seen from the Figure, the samples containing ii) spearmint oil and eugenol (63.5:36.5) and iii) eugenol were very effective at reducing the number of bacteria. The oils in these samples had specific gravities

of 0.9895 (ii) and 1.0775 (iii), respectively. Although the sample containing I) spearmint oil had some antimicrobial activity, this sample was less effective than samples containing oils ii) and iii). The specific gravity of spearmint oil (I) was 0.9508. The results for the Control show that the oil combination has little or no antimicrobial activity on its own.

Example 2

Example 1 was repeated with the following oils: iv) spearmint oil and wintergreen oil(49:51) and v) wintergreen oil. A control C2) of wintergreen oil only was also prepared. However, no parabens were included in the control.

The results are also shown in Figure 1. As can be seen from the Figure, samples containing iv) wintergreen oil and spearmint oil and v) wintergreen oil had excellent antimicrobial activity. The oils in these samples had specific gravities of iv) 1.067 and v) 1.186, respectively.

Example 3

In this example, the procedure of Example 1 was repeated to test the activity of a composition comprising methyl paraben, propyl paraben and wintergreen oil against *Enterobacter cloacae*. A composition comprising methyl paraben, propyl paraben and fennel oil was also tested. In each case, 0.117%w/v methyl paraben and 0.018%w/v propyl paraben were employed. The amount of oil in each case was 0.078%v/v.

The results are shown in Figure 2. As can be seen from the Figure, the wintergreen oil composition had better antimicrobial activity than the fennel oil composition. Wintergreen oil has a specific gravity of 1.1864, whereas fennel oil has a specific gravity of 1.001.

Example 4

The procedure of Example 3 was repeated with a culture of *Klebsiella oxytoca*. As can be seen from Figure 3, the wintergreen oil composition had better antimicrobial activity than the fennel oil composition.

Example 5

The procedure of Example 4 was repeated with a culture of *Ps. Aeruginosa*. As can be seen from Figure 4, the wintergreen oil composition had better antimicrobial activity than the fennel oil composition.

Comparative Example

In this Example, mixtures of an essential oil and methyl paraben were inoculated with a suspension of *Ps.aeruginosa* to give a final cell concentration of 2.3 x 10^8 CFU/ml. As a control, methyl paraben was also inoculated with a suspension of *Ps.aeruginosa* to give a final cell concentration of 2.3 x 10^8 CFU/ml. The methyl paraben concentration in each case was 0.46 weight %. The oils tested were orange oil and lemon oil.

As can be seen from the Table below, orange oil and lemon oil hardly potentiate the antimicrobial activity of the methyl paraben. Lemon oil has a specific gravity of 0.842 to 0.851. Orange oil has a specific gravity of 0.842 to 0.852.

Time (min) / Flavour (% v.v)	5	10	30	60	120
Orange oil (0.05)	1.65×10^8	8.17×10^7	6×10^7	1.38×10^7	5.58×10^6
Orange oil (0.02)	2.05×10^8	$1.0^7 \times 10^8$	6.17×10^7	$1.5^7 \times 10^7$	1.87×10^6
Lemon Oil (0.05)	$1.87^5 \times 10^8$	9.5×10^7	4.07×10^7	1.2×10^7	2.23×10^5
Lemon oil (0.02)	2.23×10^8	9.17×10^7	6.83×10^7	1.9×10^7	1.62×10^6

Pharmaceutical Composition

Example A

A composition containing

Sodium alginate	100g
Sodium bicarbonate	26g
Calcium carbonate	32g
Monopotassium phosphate	0.6g
Dipotassium phosphate	5.4g
Ethyl parahydroxybenzoate	2g
Butyl parahydroxybenzoate	0.2g
Sodium saccharin	1g
Spearmint Oil	0.7g
Deionised water	to 1 litre

is made up as follows

1. 917ml of deionised water are dispensed into a mixing vessel and cooled to approximately 20°C.

2. The monopotassium phosphate and dipotassium phosphate are added and stirred until dissolved.

3. The biocides, carbonates, essential oil and sweetener are added to the mixture and stirred for 5 minutes.

4. The alginate is added with stirring over a period of 3 minutes.

5. The mixture is stirred for 30 minutes (the flavour being added after 10 minutes).

6. The temperature is controlled during manufacture to 22°C (plus or minus 5°C).

CLAIMS

1. Use of an essential oil to potentiate the activity of a biocide, wherein the essential oil has a specific gravity of at least 0.89 and a dielectric constant of 1 to 20, and, preferably, wherein the essential oil does not consist of a single essential oil selected from fennel oil, anise oil, basil oil, fenchone, trans-anethole and estragol.

2. Use of an essential oil as a preservative in a liquid composition comprising a biocide, wherein the essential oil has a specific gravity of at least 0.89 and a dielectric content of 1 to 20, and, preferably, wherein the essential oil does not consist of a single essential oil selected from fennel oil, anise oil, basil oil, fenchone, trans-anethole and estragol.

3. Use as claimed in either claim 1 or 2, wherein the essential oil has a specific gravity of at least 0.990.

4. Use as claimed in any one of the preceding claims, wherein the essential oil has a dielectric constant of 2 to 18.

5. Use as claimed in claim 4, wherein the essential oil has a dielectric constant of 3 to 12.

6. Use as claimed in any one of the preceding claims, wherein the biocide is at least one biocide selected from esters of p-hydroxy benzoic acid, bronopol, chlorocresol and an isothiazole.

7. Use as claimed in any one of the preceding claims, wherein the essential oil is selected from at least one of wintergreen oil, carvone, clove oil, spearmint oil, methyl salicylate and eugenol.

8. Use as claimed in claim 7, which comprises a mixture of wintergreen oil and eugenol.

9. Use as claimed in claim 7, which comprises a mixture of wintergreen oil and spearmint oil.

10. Use as claimed in claim 7, which comprises a mixture of eugenol and spearmint oil.

11. Use as claimed in any one of the preceding claims, wherein the liquid is a pharmaceutical composition.

12. Use as claimed in any one of the preceding claims, wherein the biocide is present in an amount of 0.001 to 50 weight %.

13. Use as claimed in any one of the preceding claims, wherein the essential oil is present in an amount of 0.01 to 5 weight %.

14. Use as claimed in any one claim 11 wherein the pharmaceutical composition is in the form of an aqueous pourable liquid comprising an alginate and a bicarbonate and/or a carbonate.

15. An pharmaceutical composition comprising:

a) an alginate,

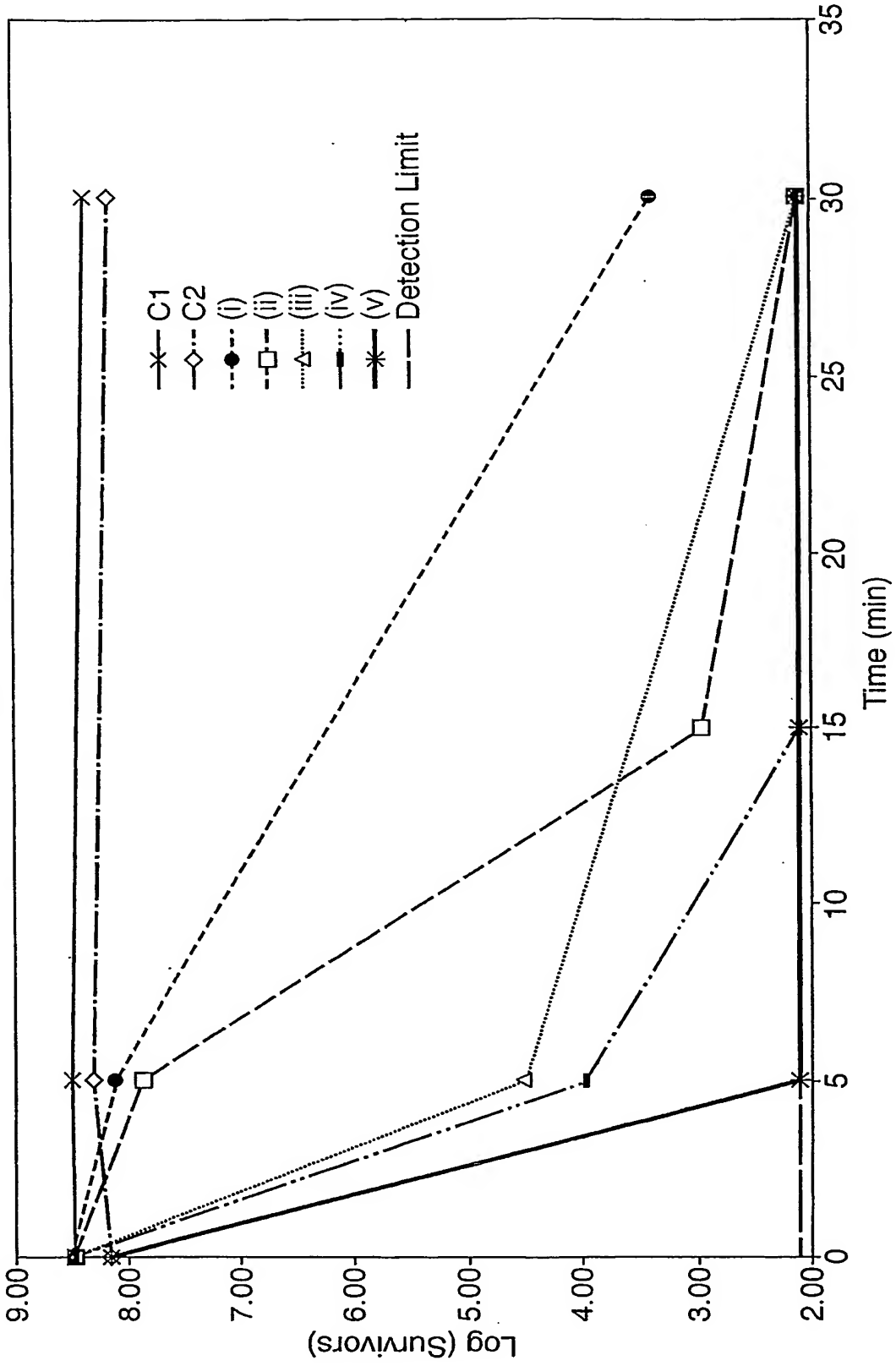
b) a bicarbonate and/or a carbonate,

c) a biocide, and

b) an essential oil,

wherein the essential oil has a specific gravity of at least 0.89 and a dielectric constant of 1 to 20, and, preferably, wherein the essential oil does not consist of a single essential oil selected from fennel oil, anise oil, basil oil, fenchone, trans-anethole and estragol.

Fig.1.


C1
C2
(i)
(ii)
(iii)
(iv)
(v)
Detection Limit
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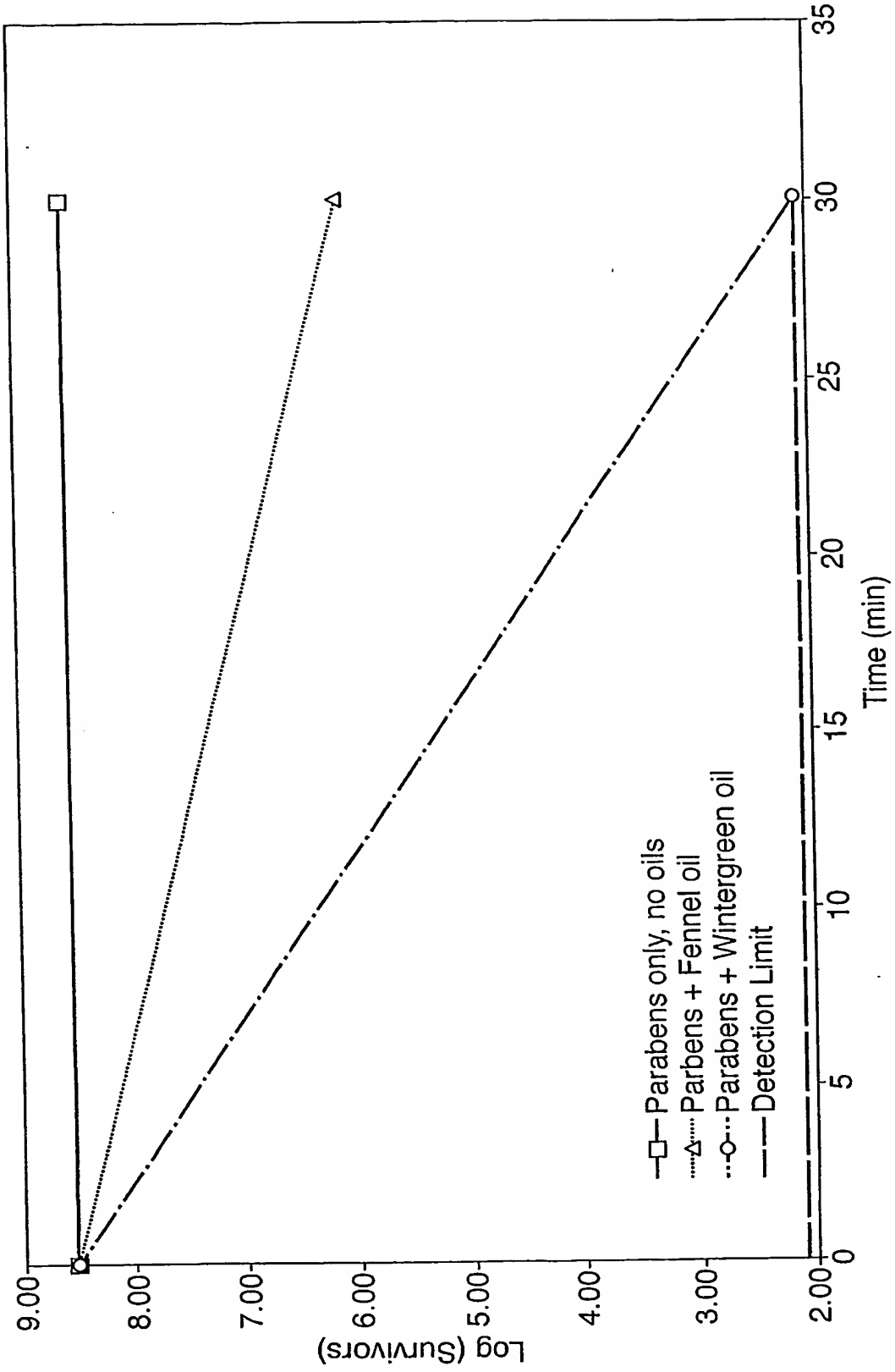
8.00
7.00
6.00
5.00
4.00
3.00
2.00
Time (min)
0
5
10
15
20
25
30
35

Fig.2.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8.00
7.00
6.00
5.00
4.00
3.00
2.00
Time (min)
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5
10
15
20
25
30
35
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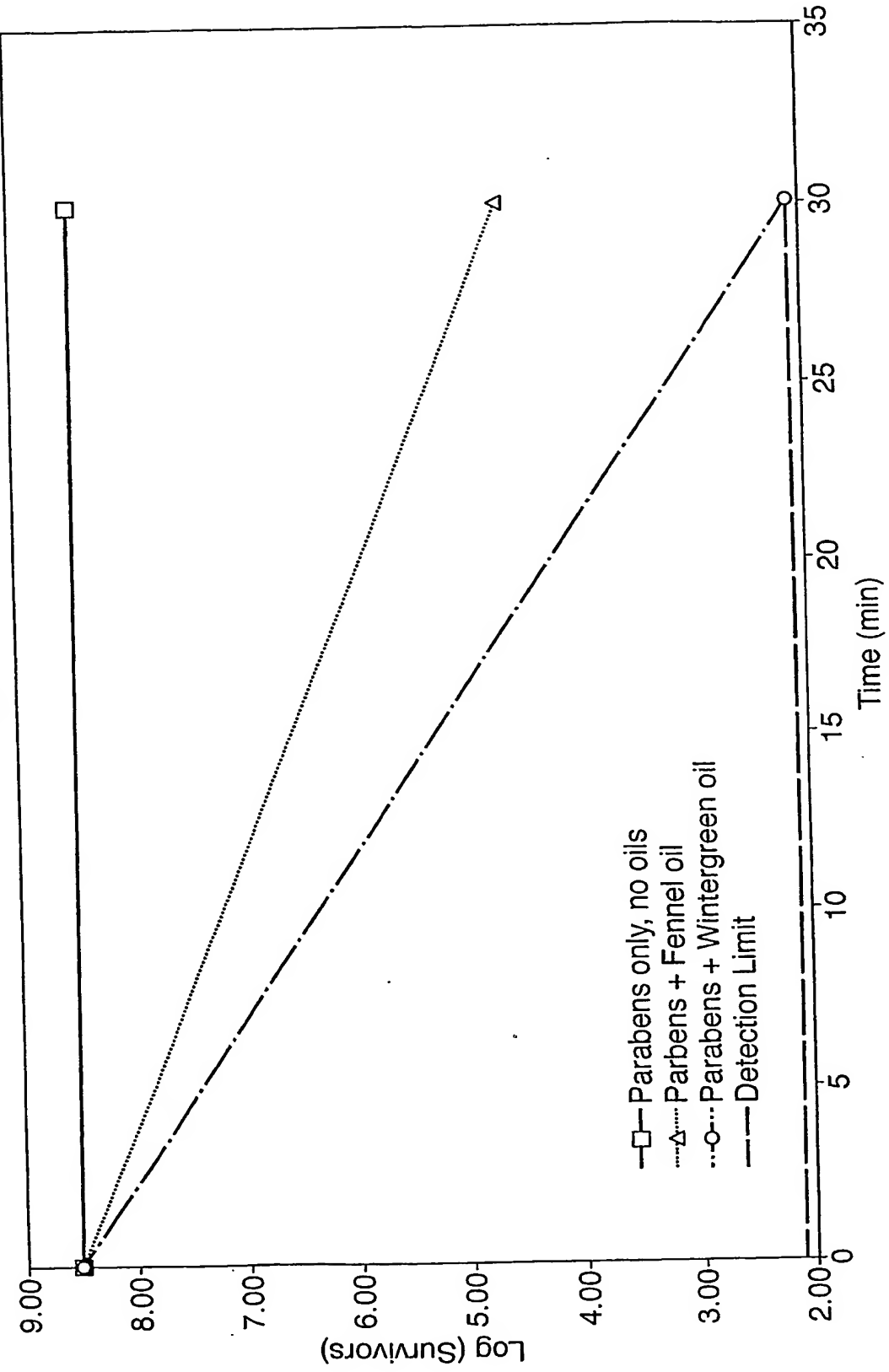
Parbens + Fennel oil
Parabens + Wintergreen oil
Detection Limit

Fig.3.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7.00
6.00
5.00
4.00
3.00
2.00
Time (min)
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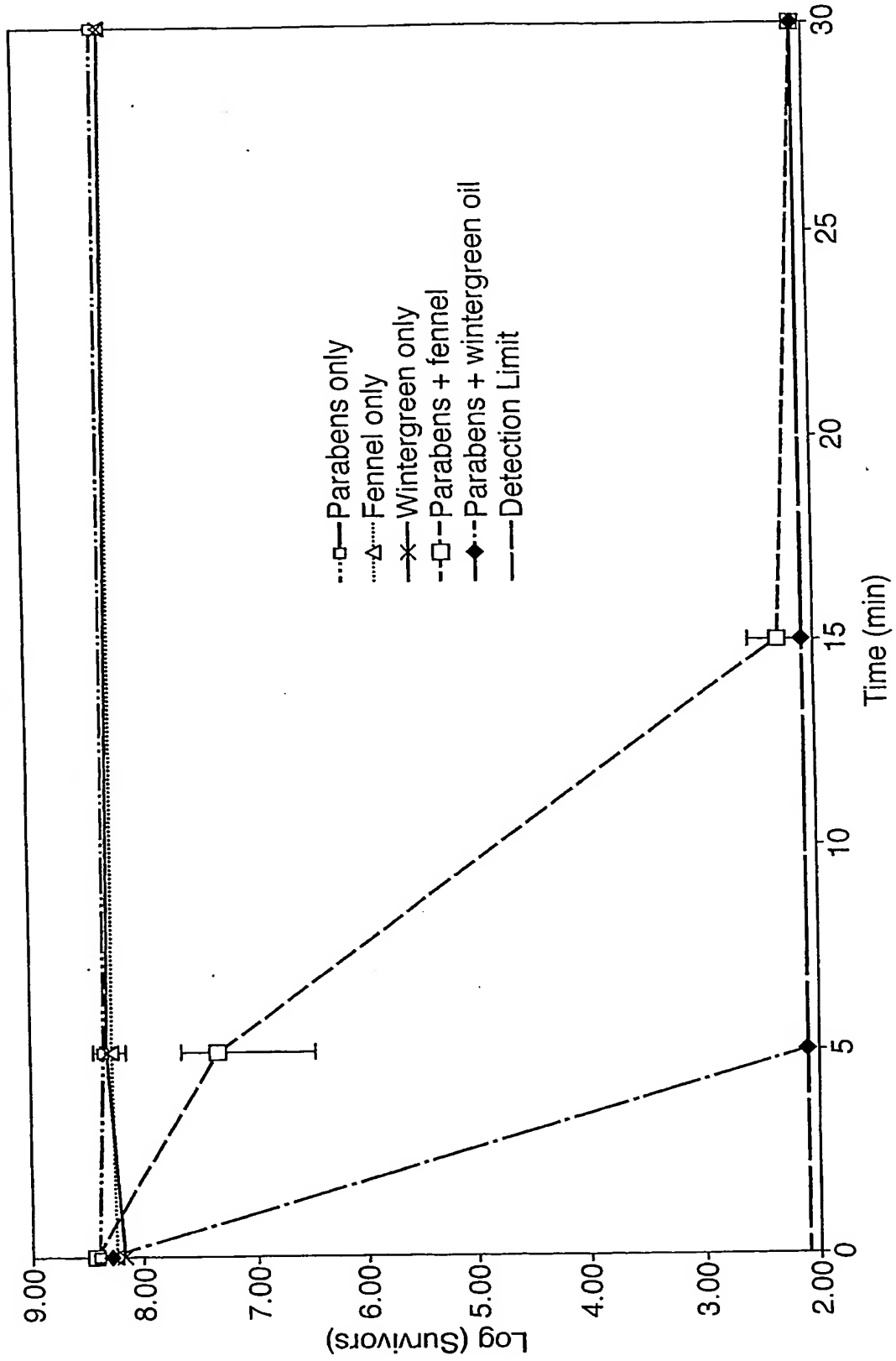
5
10
15
20
25
30
35
Parabens only, no oils
Parbens + Fennel oil
Parabens + Wintergreen oil
Detection Limit

Fig.4.


Parabens only
Fennel only
Wintergreen only
Parabens + fennel
Parabens + wintergreen oil
Detection Limit
Log (Survivors)
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8.00
7.00
6.00
5.00
4.00
3.00
2.00
Time (min)
0
5
10
15
20
25
30